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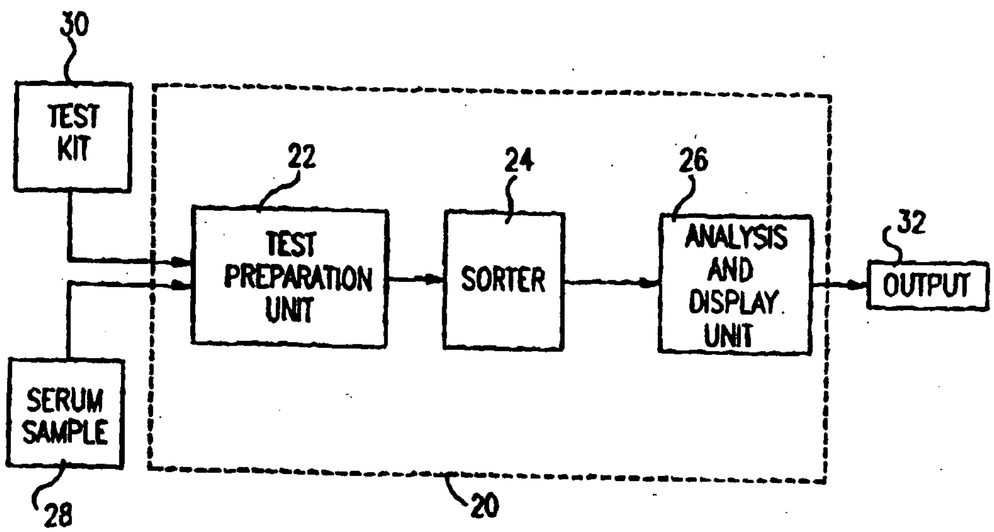

(57) Abstract

This invention discloses a test kit for multi-antigen serological diagnosis including a plurality of bead groups, each group including at least one bead, and a plurality of types of antigens, wherein each bead group has at least one common identifying physical characteristic, and wherein each type of antigen is bound to the at least one bead of one, respective, bead group. A method for assaying multiple types of antibodies in a serum sample is also disclosed.

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MULTI-ANTIGEN SEROLOGICAL DIAGNOSIS

FIELD OF THE INVENTION

The present invention relates generally to medical diagnostic testing, and specifically to apparatus and methods for detection of antibodies in serum samples.

BACKGROUND OF THE INVENTION

Methods and apparatus for immunoassay diagnostic tests are well known in the art, for example in diagnosing viral infections, auto-immune disorders and other pathologies and conditions, such as pregnancy. These tests are based on antibody-antigen interactions, wherein a serum sample taken from a subject is applied to an antigen, such as a particular protein, which reacts with a specific type of antibody in the serum that recognizes and responds to the antigen. The reacted antibody is typically bound to a labeling substance, whose concentration is then detected in order to measure the level of the specific antibody in the serum sample to be assayed. Labeling substances known in the art include, inter alia, radioisotopes (for radioimmunoassay), conjugated enzymes (for ELISA - enzyme-linked immunoassay), and fluorescent labels.

Immunoassay tests must frequently be performed on a serum sample for more than one type of antigen. Generally in these cases the test is performed separately for each specific antigen-antibody reaction, for example, for a single strain of influenza virus. The test must then be repeated for each different antigen of interest.

Double antigen testing, wherein a different label is used for each antigen, is also known in the art. Such testing is useful for direct detection of antigens, but is not practical for antibody detection. In this method, antibodies of two different types, each type labeled with a different, respective fluorescent, isotopic or other marker, are added to a sample containing antigens. The concentrations of the two types of antigens are detected, for example by observing fluorescence in two different respective colors using a spectrometer. This method, however, is practically impossible to implement when more than two antigens are to be detected.

A number of methods have been developed for performing multiple single-antigen assays together on a given serum sample. The antigens in these assays are preferably bound to solid-phase substrates, for more convenient handling. Such assays are performed, for example, using the EL312e reader and EL404 washer made by Bio-Tek Instruments, Inc., of Winsooki, Vermont, USA, or the MAGIA series of instruments made by E. Merck Diagnostica, Darmstadt, Germany. Test kits using these instruments typically include a test substrate having multiple plastic wells, wherein a different antigen to be tested for is bound to each of the wells. Up to 96 wells, each with a different antigen, may be used on a single substrate.

Alternatively, plastic or magnetic beads may be used to bind the antigens, as described, for example, by D. Leahy, et al., in Transfusion 32 (1992), pages 548-553, which is incorporated herein by reference. As described in this article, the beads are of uniform size, and all the beads in a given test tube or test cell are bound to antigens of a single type.

Petts, et al., in Eur. J. Clin. Microbiol. Infect. Dis. 7 (1988), pages 34-39, and Hadfield, et al., in Journal of Immunological Methods 97 (1987), pages 153-158, describe other diagnostic techniques, in which two or more different antigens are bound to beads of respective, different colors. When antibodies in a sample react with the antigens, the beads to which these antigens are bound form a dense precipitate. The color of the precipitate is thus indicative of the type of antibodies in the sample. This type of test is useful in qualitative assays, for example, in identifying which single type of a group of types of antibodies is present in the sample, but it is not generally practical for multiple-antibody diagnosis or for quantitative determinations.

Fluorescence-activated cell sorting (FACS) devices and methods are well known in the art. FACS is performed using flow cytometry methods and apparatus, such as the FACStar PLUS™ family of instruments, manufactured by Becton Dickinson Immunocytometry Systems, of San Jose, California. In flow cytometry, cells in a stream of fluid are made to pass through a laser beam, which causes them to fluorescently emit and/or scatter light. The intensity of this emitted or scattered light signal from each individual cell is analyzed. In FACS, this signal is then used to cause an electrical charge, dependent on the signal, to be applied to a droplet of fluid containing the cell. A

system of electrical deflection plates downstream causes the droplets to be sorted and collected according to the signals they produced.

FACS is commonly used to sort or analyze cells on the basis of their surface antigens, by first treating them with an antibody that has been conjugated to a fluorescent probe molecule. In order to test and calibrate the FACS apparatus, beads of substantially the same size as cells are inserted into the apparatus for processing, as described by Dulling and Waldschmidt in ISAC XVI Cytometry Supplement 6 (1993), page 47; by Bell and Shenton, in ISAC XVII Cytometry Supplement 7 (1994), page 38; and by Mathai, et al., in ISAC XVII Cytometry Supplement 7 (1994), page 38, which are incorporated herein by reference.

Additionally, the use of beads/particles for serological analysis has been described in the European Patent Application No. 93110399.8, titled "Immunoassay Using Microparticles Containing Different Detectable Substances", applicant Becton, Dickson & Company, and inventors T. J. Mercolino, J.H. Hasskamp and E.C. McFarland, filed June 30, 1993 and published in Bulletin 94/01, Publication No. 0577092A2 on January 5, 1994. This patent application describes a method for quantifying the amount of complex antigen/antibody used in serological diagnosis. In this method described, particles are used as carriers of the colored tracer and at the same time as markers for the antigen.

Furthermore, the review articles "New Developments in Particle-Based Immunoassay: Introduction", by L.B. Bangs and published in Pure and Applied Chemistry, Vol. 68(10) pp. 1873-9, 1996, and "Diagnostic Application of Microspheres", by L.B. Bangs, published in Liquid- and Surface-Borne Particle Measurement Handbook, Chapter 15, pp. 687-707, Editors J.Z. Knapp, T.A. Barber and A. Lieberman, published by Marcell Dekker, Inc., New York, describe the use of beads/particles in multi-antigen diagnosis. The review articles suggest the use of multi-antigen diagnosis for both protein diagnostics and nucleic acid diagnostics.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a convenient method of assaying multiple antibodies simultaneously in a single sample.

In one aspect of the present invention, the multiple antibody assay is performed using methods of flow cytometry.

It is a further object of the present invention to provide apparatus for assaying multiple antibodies simultaneously in a single sample and a method for controlling thereof.

In one aspect of the present invention, this apparatus operates in conjunction with a flow cytometry system.

Yet another object of the present invention is to provide a test kit for preparation of a sample for assaying antibodies for multiple antigens simultaneously.

In preferred embodiments of the present invention, a multi-antigen test kit comprises a multiplicity of beads, having a plurality of sizes and/or shapes and/or colors, wherein the sizes and/or shapes and/or colors are chosen so that the beads may be sorted according to their respective shapes, sizes and/or colors. Preferably the sizes of the beads are on the order of the size of cells, and they are sorted by fluorescence-activated cell sorting (FACS).

The term "beads" in the context of the present invention is taken to mean miniature pellets or particles, which may be of any desired shape. In one preferred embodiment of the present invention, the beads are substantially spherical.

Additionally, throughout the specification and claims of the present patent application, the term "color" refers to the broader meaning of wavelength, the intensity of spectral emissions, absorbances and excitations.

Preferably the test kit includes a plurality of different types of antigens, wherein each antigen type is associated with a respective size, shape and/or color, and wherein the specific antigen is bound to all beads in the kit having substantially the same size, shape and/or color. It will be appreciated that the beads are many times larger than the antigens and the antibodies reacting therewith, so that the sizes and shapes of the beads remain substantially unchanged during use of the test kit.

Furthermore, in preferred embodiments of the present invention, the multiplicity of antigen-bound beads are mixed with a serum sample, causing specific antibodies present in the sample to bond to corresponding specific antigens. The multiplicity of beads to which the sample has been applied are then developed, using methods known in the art, so that a fluorescent-labeled molecule is conjugated or bound to each of the bonded antibodies. The developed beads are passed through flow cytometry apparatus, which sorts the beads according to their sizes and/or shapes and/or color and analyzes the fluorescent light signal received from each of the beads. It will be appreciated that in this manner, the beads are sorted into groups, each group corresponding to a different antigen, whereby the fluorescent light signals received from each group are indicative of the presence and concentration level of the specific antibody in the sample associated with the single specific antigen.

In accordance with preferred embodiments of the present invention, the flow cytometry apparatus is adapted to provide a data readout that includes simultaneous assays of the levels of all the specific antibodies in the sample for which specific antigens were provided. The flow cytometry apparatus preferably applies calibration standards and thresholds, as are known in the art, to the analysis of the data. Preferably the data output includes a list of pathologies, disorders and/or other medical conditions, wherein each of the listed pathologies, disorders and conditions is followed by an indication of its presence or absence, and/or by a measure of the concentration of antibodies in the sample associated with the pathology, disorder or condition. The data output may include a histogram or other statistical analysis.

In preferred embodiments of the present invention, the test kit, flow cytometry apparatus and method are suitable for assaying viral agents, such as HIV, hepatitis, herpes, influenza and other agents known in the art.

Additionally or alternatively, the test kit, flow cytometry apparatus and method are suitable for diagnosing autoimmune disorders, such as systemic lupus erythematosus, or myasthenia gravis, or, in still other preferred embodiments, for analyzing physiological conditions, such as pregnancy, cancer, respiratory virologies, infections endangering pregnant women, kidney disorders, liver disorders, as well as pathology, epidemiology.

There is therefore provided, in accordance with a preferred embodiment of the present invention, a test kit for multi-antigen serological diagnosis including a plurality of bead groups, each group including at least one bead; and a plurality of types of antigens, wherein each bead group has at least one common identifying physical characteristic, and herein each type of antigen is bound to the at least one bead of one, respective, bead group.

Preferably the at least one identifying physical characteristic includes bead size or bead shape or bead color, and the largest dimension of each bead is between 0.5 and 10 micrometers. Preferably at least some of the beads are substantially spherical.

Preferably the plurality of groups of beads includes at least three groups of beads, and the plurality of types of antigens includes at least three different, respective, types of antigens.

Preferably at least one of the plurality of types of antigens is adapted for assaying anti-viral antibodies. Additionally or alternatively, preferably at least one of the plurality of types of antigens is adapted for detecting autoimmune disorders, cancer markers, respiratory virologies, infections endangering pregnant women, pathology, epidemiology, kidney disorders, liver disorders.

There is further provided, in accordance with a preferred embodiment of the present invention, apparatus for multi-antigen serological diagnosis of a serum sample, including a test kit as described above; and a sorter, adapted to sort the beads according to their respective, identifying, physical characteristics.

Preferably the sorter sorts the beads according to their sizes and/or shapes and/or color.

Preferably the sorter includes fluorescence-activated cell sorting apparatus, which sorts the beads according to fluorescent light emission therefrom. Preferably the sorter further sorts the beads according to a physical characteristic indicative of presence or substantial absence of predetermined antibodies on the beads.

Preferably the apparatus includes a processor, which provides an output responsive to the presence or substantial absence of predetermined types of antibodies in the sample; a display, which displays information relating to the sample based on said

output; and a printer, which prints information relating to the sample based on said output.

Preferably the apparatus also includes a preparation unit, which prepares the test kit for sorting. Preferably the preparation unit includes an applicator which applies the sample to the test kit, and a developer which develops a predetermined marker onto at least some of the beads that have antibodies reacted with the antigens thereon.

There is also provided, in accordance with a preferred embodiment of the present invention, a method for assaying multiple types of antibodies in a serum sample, including the following steps:

providing a plurality of beads;

binding a plurality of types of antigens to the beads;

applying a serum sample to the beads;

developing the beads with a marker;

sorting the developed beads, so as to generate a bead count;

classifying the levels of one or more types of antibodies in the sample using the bead count.

Preferably providing a plurality of beads includes providing a plurality of groups of beads, all beads in each group having a common identifying physical characteristic. Preferably each type of antigen is bound to substantially all the beads in one, respective, group of beads. Preferably beads from different groups are mixed according to the types of antibodies to be assayed, and beads from at least three different groups are mixed.

Preferably developing the beads includes conjugating a fluorescent marker to at least some of the antibodies that have reacted with antigens bound to the beads. Preferably a plurality of different fluorescent markers, respectively, are conjugated to a plurality of different types of antibodies.

Preferably sorting the beads includes sorting the beads by size, shape and/or color.

Preferably sorting the developed beads further includes:

irradiating the beads with laser radiation;

receiving light signals from the beads;

identifying the beads according to the light signals received therefrom; and

counting beads from which substantially similar light signals were received.

Preferably receiving light signals from the beads includes receiving scattered light signals and/or receiving fluorescent emission light signals.

Preferably classifying the levels of one or more types of antibodies in the sample includes applying thresholds to the bead count.

Preferably classifying the levels of one or more types of antibodies in the sample further includes determining the presence or substantial absence of the one or more types of antibodies and/or determining the respective concentrations of the one or more types of antibodies.

Preferably classifying the levels of one or more types of antibodies in the sample includes classifying the levels of anti-viral antibodies and/or of antibodies indicative of autoimmune disorders, cancer markers, respiratory virologies, infections endangering pregnant women, pathology, epidemiology, kidney disorders, liver disorders.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be more fully understood from the following detailed description of the preferred embodiments thereof, taken together with the drawings in which:

Fig. 1 is schematic illustration of apparatus for analyzing antibody levels in a serum sample, in accordance with a preferred embodiment of the present invention;

Fig. 2 is a schematic illustration of a test preparation unit for preparation of samples for analysis, in accordance with the preferred embodiment of the present invention shown in Fig. 1;

Fig. 3A is a schematic illustration of beads coated with specific antigens, in accordance with a preferred embodiment of the present invention;

Fig. 3B is a schematic illustration of the beads of Fig. 3A, mixed together in a test kit, in accordance with a preferred embodiment of the present invention;

Fig. 3C is a schematic illustration of the test kit of Fig. 3B, after application of the serum sample, in accordance with a preferred embodiment of the present invention;

Fig. 3D is a schematic illustration of color development of the test kit and serum sample of Fig. 3C, in accordance with a preferred embodiment of the present invention;

Fig. 4 is a schematic illustration of a sorter for sorting beads and receiving fluorescence signals from samples for analysis, in accordance with a preferred embodiment of the present invention shown in Fig. 1;

Fig. 5 is a table, representing schematically sample results of the operation of the reading unit;

Fig. 6 is a schematic illustration of an analysis and display unit for analysis and presentation of data, in accordance with a preferred embodiment of the present invention shown in Fig. 1; and

Fig. 7 is a schematic illustration of a Sample output of the Analysis and Display unit.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Fig. 1 illustrates schematically apparatus 20 for multi-antigen serological diagnosis on a single sample, in accordance with a preferred embodiment of the present invention. Apparatus 20 preferably comprises a test preparation unit 22, a sorter 24 and an analysis and display unit 26. As will be described below in greater detail, test preparation unit 22 receives a serum sample 28 and a suitably prepared test kit 30. The test kit and serum combination is processed as illustrated in Fig. 1 and in subsequent figures, and outputs test results 32. These results preferably comprise data relating to the levels of specific antibodies detected in serum sample 28.

Fig. 2 schematically illustrates test preparation unit 22, which preferably comprises a mixer 40, for preparing test kit 30; applicator 42, for applying serum sample 28 to the prepared test kit; and developer 44, for color development of the test kit after application of the serum sample. Mixer 40, applicator 42 and developer 44 are preferably automated laboratory devices of types known in the art, and preferably operate under the control of a robot 46, likewise of a type known in the art. Alternatively, some or all of the operations of test preparation unit 22 may be performed manually by a laboratory technician. After these operations are completed, the test kit with serum sample is ready for transfer to sorter 24.

Figs. 3A-3D illustrate more clearly processes executed by test preparation unit 22. As shown in Fig. 3A, test kit 30 comprises a multiplicity of beads 50, 52, 54, having a plurality of different sizes, and grouped initially according to these sizes. Preferably the beads have dimensions in the range 0.5 to 10 micrometers and are made of plastic, for example polypropylene, polystyrene, or latex, as described in the references cited above, or other polymers or particles known in the art. It will be understood that although, in accordance with one preferred embodiment of the present invention, beads 50, 52 and 54 shown in Figs. 3A-3D are spherical, in other preferred embodiments of the present invention, the beads may have different shapes and colors, and are grouped and sorted according to their shape or size or color or combination thereof.

A first type of antigen 56 is bound, using methods known in the art, to substantially all beads 50, which are all of the same size. Similarly, a second type of antigen 58 is bound to substantially all beads 52, and a third type of antigen 60 is bound

to substantially all beads 54. It will be understood that although for clarity of explanation, the figures show only three different sizes and corresponding antigens, in preferred embodiments of the present invention, the number of different sizes and shapes and colors can be much larger. Thus, in preferred embodiments of the present invention, test kit 30 can include tens or hundreds of different types of antigens, each type bound to beads of a different size and/or shape and/or color.

As shown in Fig. 3B, the beads of different sizes from test kit 30 are mixed together by mixer 40 (shown in Fig. 2) in a single test cell 64, and the serum sample 28 and the set of mixed beads 50, 52, 54, are applied to the applicator 42. It will be appreciated that each of antigens 56, 58 and 60 of the serum sample 28 is adapted to react with a corresponding specific antibody 66, 68 or 70, respectively, which may be present in serum sample 28. Preferably beads 50, 52 and 54 are selected and mixed together in preparation for analysis, based on the specific antibodies whose levels are to be analyzed. Alternatively, test cell 64 may be prepared by the manufacturer with test kit 30 in pre-mixed form.

Referring now to Fig. 3C, it will be seen that when serum sample 28 is applied by applicator 42 (shown in Fig. 2) to the contents of test kit 30, antibodies 66 react with antigens 56 on beads 50, and antibodies 68 react with antigens 58 on beads 52. In this example, however, antibodies 70 are not present in the serum sample, and therefore, antigens 60 on beads 54 remain unreacted.

Finally, as shown in Fig. 3D, developer 44 (shown in Fig. 2) biochemically develops the kit to which serum sample 28 has been applied, using methods and materials known in the art, wherein reacted antibodies are conjugated to fluorescent dye markers, for example FITC (fluorescein) or rhodamine. Thus, after development, beads having antibody-reacted antigens, such as antigen 56, will produce fluorescence of a predetermined color when illuminated by suitable laser light, while beads having non-reacted antigens of the same type, such as antigen 56A, will not provide fluorescence of this color. Based on the difference in fluorescence, beads of substantially the same size, such as beads 50 and 50A, are distinguishable by color as to whether antibody 66 has reacted with an antigen 56 thereon. Distinguishing these beads 50 and 50A would otherwise be very difficult, because antigens 56, 58 and 60 and antibodies 66, 68 and 70

are typically smaller than the beads by several orders of magnitude. Antigens 56, 58 and 60 are shown to be comparable in size to the beads in Figs. 3A-3D for demonstrative simplicity only.

Although generally all reacted antibodies are marked with the same color, in some preferred embodiments of the present invention, different types of reacted antibodies are marked with different color dyes. These embodiments are useful when more than one type of antibody may react with a given antigen, for example antibodies IgG, IgM and IgE, as are known in the art. In this case the different colors associated respectively with the different antibodies are used to determine their respective concentrations in the sample.

Fig. 4 schematically illustrates sorter 24, which sorts the colored beads 50, 52 and 54 following development by developer 44. In the preferred embodiment of the present invention shown in Fig. 4, sorter 24 includes a fluorescence-activated cell sorting (FACS) system, such as the FACStar PLUS system, manufactured by Becton Dickinson, or any other type of FACS system known in the art. In this system, beads 50, 52 and 54 are mixed with a sheath fluid 72 and expelled through a nozzle 74, which directs them through a laser beam 75, preferably in the 400-700 nm range of wavelength, produced by a laser 76. As each bead 50, 52, 54 passes through the laser beam, the amount and pattern of light that the beads 50, 52, 54 scatter is measured by a scattering detector 77. This measurement is used by processor 79 to determine the size and/or shape and/or color of the bead, using methods known in the art, and thus to sort the beads by size and/or shape and/or color. Since each different size or shape bead is bound to a different specific antigen, determining the beads' sizes allows sorting of the different types of antigens.

Furthermore, when laser beam 75 strikes a bead, conventional fluorescent marker chemicals on the bead emit a fluorescent light signal, which is detected by fluorescence detector 78. The level of this signal is proportional to the number of antibodies that have reacted with antigens on the bead. Thus, the fluorescent signal received from a bead of a given size, corresponding to a specific antigen, is responsive to the concentration level of the specific antibody in sample 28 corresponding to the antigen. This signal is used by processor 79 to determine the concentration of antibodies on the bead.

In a conventional FACS system, the FACS system is calibrated using beads of predetermined known sizes and shapes. Such calibration of FACS systems using beads is known in the art for the purposes of calibrating the systems to sort biological cells of comparable size and/or shape.

In a preferred embodiment of the present invention, the identity of an antigen, bound to a bead of known size, shape, and color (wavelength and brightness) is determined in real time. The method for controlling the identification of the antigen is performed in real time from the size, shape and intensity of the 5 out of 6 colors read by the FACS system. The sixth color, FL1, of the antigen is used for determining the antibody binding.

In the real time antigen identification method, a set of beads of known size, shape and color, and with various FL1 intensities, preferably different from the size, shape, and color of the antigens to be identified, are introduced into the apparatus. Using this group of the beads, the (0,0,0) coordinates of the matrix, which describes the parameters of the antigen are determined. The brightness and intensity of the scattered laser signal received are dependent on the ratio of antigens/antibodies in the sample. The parameters, namely size, shape, and color, of the antibody are determined from the fluorescent signal emitted by the fluorescent chemical marker. From these measured data, the size, shape, and color of the antigen are determined. These parameters allow the identification of the antigen in real time.

By using substantially similar beads in all test kits, it is possible to standardize the method of identifying an antigen in real time.

Preferably the calibration includes determining one or more threshold values of size and color. During sorting of the processed test kits, the predetermined threshold values are applied to data received by processor 79, in order to produce an output indicative of the levels of antibodies in the sample. This output may be in the form of a bargraph 80, as shown schematically in Fig. 5.

Referring now to the sample results shown in Fig. 5, it is noted that one bead of "size 1" emitted a signal with a color "(+)," indicating that antibody 66 has reacted with antigen 56 on bead 50, as shown in Fig. 3D. However, since no antibodies have reacted with antigens on other beads of "size 1," such as antigen 56A on bead 50A, two beads of

"size 1" were thus found to have emitted signals of color "(-)," indicating that the antigen-antibody reaction has not occurred. The histogram values for beads 52 and beads 54, listed respectively as "size 2" and "size 3" in Fig. 5, are similarly derived.

It will be appreciated that although Fig. 5 shows only two values of color, (+) and (-), corresponding to whether the fluorescent signals received are greater or less than a given threshold value, in other preferred embodiments of the invention, multiple thresholds may be set, corresponding to multiple different levels of fluorescent emission.

The output data from sorter 24 are then analyzed and displayed by analysis and display unit 26, shown schematically in Fig. 6. Unit 26 preferably comprises output data processor 90, which receives data from processor 79 (shown in Fig. 4). Processor 90 pools the data received regarding the sizes of the beads, the shapes of the beads, and the color of the beads, and matches the output data with corresponding specific antigen-antibody reactions. The fluorescent signal data are then classified in accordance with predetermined standards for classifying antigen-antibody reactions, as are known in the art. These standards may be preset by the manufacturer of the apparatus, or, alternatively, they may be set by the user in accordance with specific test requirements. Typically, the standards are used to classify the antibody level corresponding to each specific antigen as zero, high or low, although other standards classifications may alternatively be used. Classification results 32 are output to display 92 and printer 94, for example, in the form of tables and/or graphs.

Fig. 7 shows a typical output table 100, in accordance with a preferred embodiment of the present invention, comprising classification results 32 for samples taken from five patients and tested for five different viruses. As illustrated by Fig. 7, in preferred embodiments of the present invention, the test kit, flow cytometry apparatus and diagnostic methods executed thereby are adapted for assaying viral agents, such as HIV, hepatitis, herpes, influenza and other agents known in the art.

In other preferred embodiments of the present invention, the test kit, flow cytometry apparatus and diagnostic methods executed thereby are adapted for diagnosing autoimmune disorders, such as systemic lupus erythematosus, or myasthenia gravis, or, in still other preferred embodiments, for analyzing physiological conditions, such as pregnancy such as pregnancy, cancer, respiratory virologies, infections

endangering pregnant women, kidney disorders, liver disorders, as well as pathology, epidemiology.

The diagnostic methods described here may be executed using any suitable circuitry and/or software incorporated, inter alia, in processor 79 and processor 90.

It will be appreciated that the preferred embodiments described above are cited by way of example, and the full scope of the invention is limited only by the following claims:

CLAIMS

1. A test kit for multi-antigen serological diagnosis comprising:

a plurality of bead groups, each group including at least one bead; and

a plurality of types of antigens,

wherein each bead group has at least one common identifying physical characteristic, and

wherein each type of antigen is bound to the at least one bead of one, respective, bead group.

2. A test kit in accordance with claim 1, wherein the at least one identifying physical characteristic is selected from the group consisting of bead size, bead shape, and bead color.

3. A test kit in accordance with claim 1 or claim 2, wherein at least some of the beads are substantially spherical.

4. A test kit in accordance with claim 1 or claim 2, wherein the largest dimension of each bead is between 0.5 and 10 micrometers.

5. A test kit in accordance with claim 1 or claim 2, wherein the plurality of groups of beads comprises at least three groups of beads and wherein the plurality of types of antigens comprises at least three different, respective, types of antigens.

6. A test kit in accordance with claim 1 or claim 2, wherein at least one of the plurality of types of antigens is adapted for assaying at least one of anti-viral antibodies, autoimmune disorders, cancer markers, respiratory virologies, infections endangering pregnant women, pathology, epidemiology, kidney disorders, liver disorders.

7. A test kit in accordance with claim 1 or claim 2, wherein at least one of the plurality of types of antigens is adapted for detecting at least one of anti-viral antibodies, autoimmune disorders, cancer markers, respiratory virologies, infections endangering pregnant women, pathology, epidemiology, kidney disorders, liver disorders.

8. Apparatus for multi-antigen serological diagnosis of a serum sample, comprising:

a test kit in accordance with any of the preceding claims; and

a sorter, adapted to sort the beads according to their respective, identifying, physical characteristics.

9. Apparatus in accordance with claim 8 wherein the sorter sorts the beads according to a characteristic selected from the group consisting of bead size, bead shape, and bead color.

10. Apparatus in accordance with claim 8 or claim 9 wherein the sorter comprises fluorescence-activated cell sorting apparatus which sorts the beads according to fluorescent light emission therefrom.

11. Apparatus in accordance with claim 8 or claim 9 wherein the sorter further sorts the beads according to a physical characteristic indicative of presence or substantial absence of predetermined antibodies on the beads.

12. Apparatus in accordance with claim 8 or claim 9, and comprising a processor, which provides an output responsive to the presence or substantial absence of predetermined types of antibodies in the sample.

13. Apparatus in accordance with claim 8 or claim 9 and comprising a display, which displays information relating to the sample based on said output.

14. Apparatus in accordance with claim 8 or claim 9 and comprising a printer, which prints information relating to the sample based on said output.

15. Apparatus in accordance with claim 8 or claim 9 and comprising a preparation unit which prepares the test kit for sorting.

16. Apparatus in accordance with claim 15 wherein the preparation unit comprises an applicator which applies the sample to the test kit.

17. Apparatus in accordance with claim 15 wherein the preparation unit comprises a developer which develops a predetermined marker onto at least some of the beads that have antibodies reacted with the antigens thereon.

18. A method for assaying multiple types of antibodies in a serum sample, comprising:

providing a plurality of beads;

binding a plurality of types of antigens to the beads;

applying a serum sample to the beads;

developing the beads with a marker;

sorting the developed beads, so as to generate a bead count;

classifying the levels of one or more types of antibodies in the sample using the bead count.

19. A method in accordance with claim 18 wherein providing a plurality of beads comprises providing a plurality of groups of beads, all beads in each group having a common identifying physical characteristic.

20. A method in accordance with claim 19 wherein binding a plurality of types of antigens to the beads comprises binding each type of antigen to substantially all the beads in one, respective, group of beads.

21. A method in accordance with claim 19 or 20 comprising mixing beads from different groups according to the types of antibodies to be assayed.

22. A method in accordance with claim 21 wherein mixing beads comprises mixing beads from at least three different groups.

23. A method in accordance with any of claims 18 -20 wherein developing the beads comprises conjugating a fluorescent marker to at least some of the antibodies that have reacted with antigens bound to the beads.

24. A method in accordance with claim 23 wherein developing the beads comprises conjugating a plurality of different fluorescent markers, respectively, to a plurality of different types of antibodies.

25. A method in accordance with any of claims 18 - 20 wherein sorting the beads includes sorting the beads according to a characteristic selected from the group consisting of bead size, bead shape, and bead color.

26. A method in accordance with any of claims 18 - 20 wherein sorting the developed beads comprises:

irradiating the beads with laser radiation;

receiving light signals from the beads;

identifying the beads according to the light signals received therefrom; and

counting beads from which substantially similar light signals were received.

27. A method in accordance with claim 26, wherein receiving light signals from the beads includes receiving scattered light signals.

28. A method in accordance with claim 26, wherein receiving light signals from the beads includes receiving fluorescent emission light signals.

29. A method in accordance with any of claims 18 - 20 wherein classifying the levels of one or more types of antibodies in the sample comprises applying thresholds to the bead count.

30. A method in accordance with any of claims 18 - 20 wherein classifying the levels of one or more types of antibodies in the sample comprises determining the presence or substantial absence of the one or more types of antibodies.

31. A method in accordance with any of claims 18 - 20 wherein classifying the levels of one or more types of antibodies in the sample comprises determining the respective concentrations of the one or more types of antibodies.

32. A method in accordance with any of claims 18 - 20 wherein classifying the levels of one or more types of antibodies in the sample comprises classifying the levels of at least one of anti-viral antibodies, autoimmune disorders, cancer markers, respiratory virologies, infections endangering pregnant women, pathology, epidemiology, kidney disorders, liver disorders.

33. A method in accordance with any of claims 18 - 20 wherein classifying the levels of one or more types of antibodies in the sample comprises classifying the levels indicative of at least one of anti-viral antibodies, autoimmune disorders, cancer markers, respiratory virologies, infections endangering pregnant women, pathology, epidemiology, kidney disorders, liver disorders.

FIG. 1

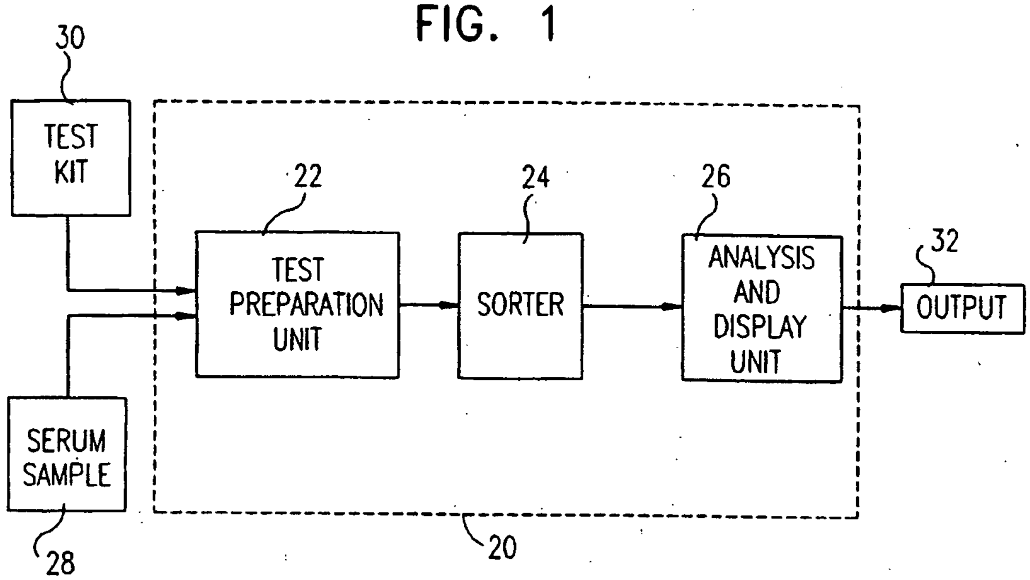
30
TEST KIT
22
TEST PREPARATION UNIT
24
SORTER
26
ANALYSIS AND DISPLAY UNIT
32
OUTPUT
SERUM SAMPLE
28
20

FIG. 2

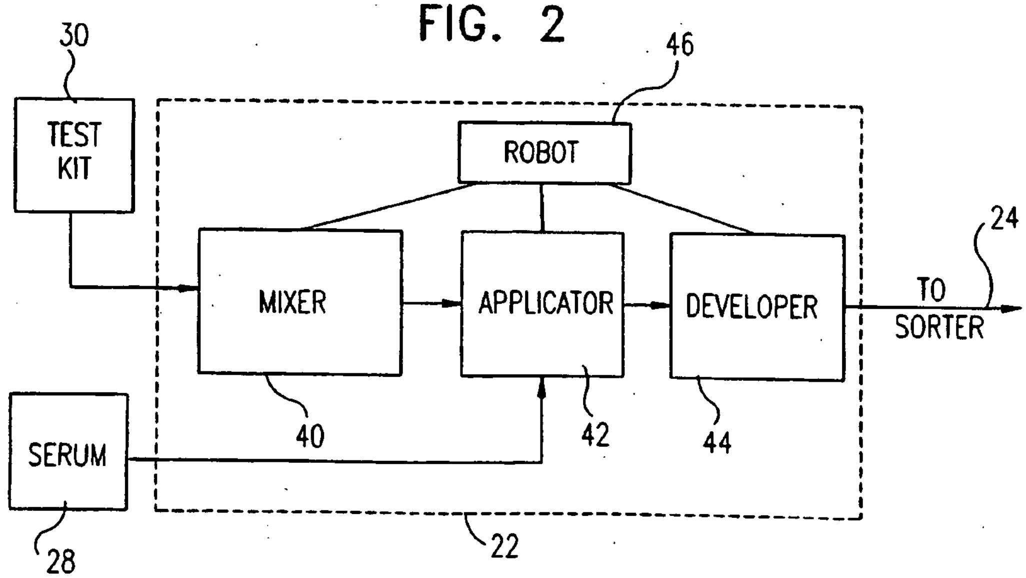
30
TEST KIT
46
ROBOT
MIXER
APPLICATOR
DEVELOPER
TO SORTER
24
40
42
44
SERUM
28
22

FIG. 3A

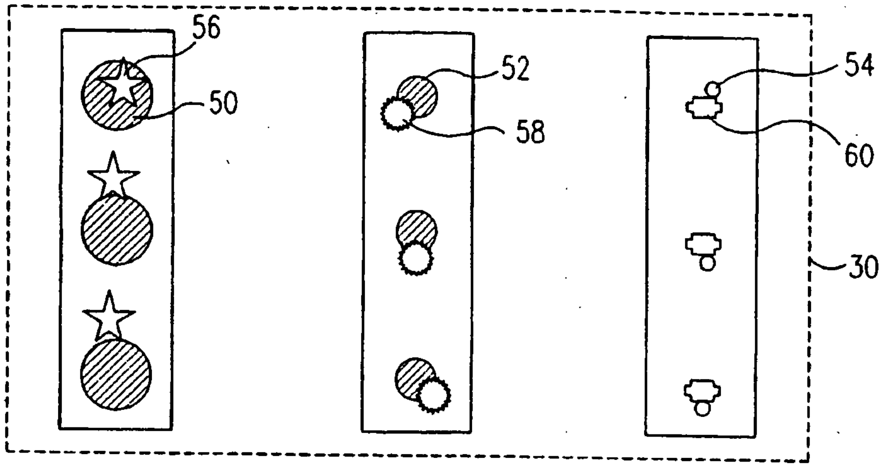

FIG. 3B

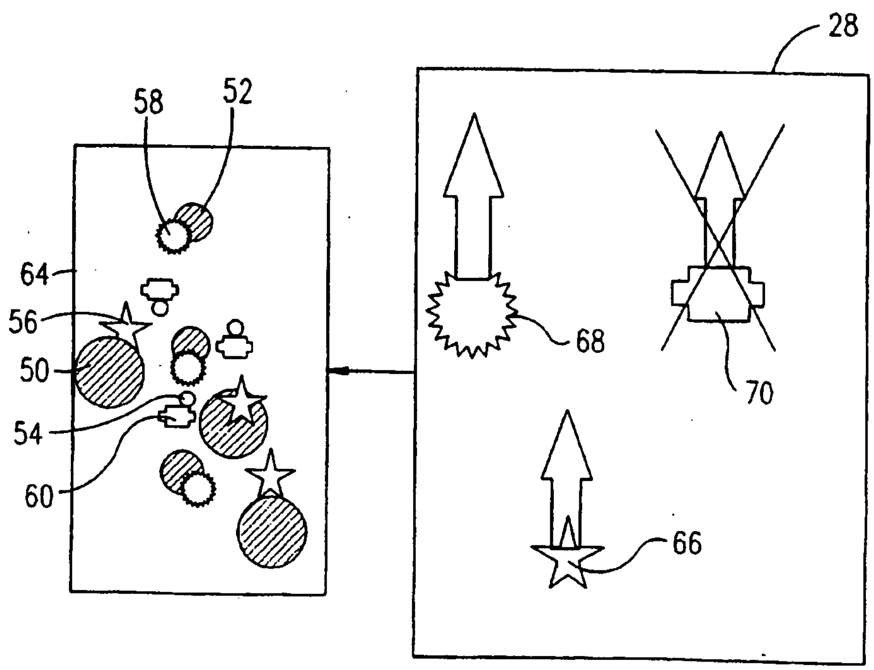

FIG. 3C

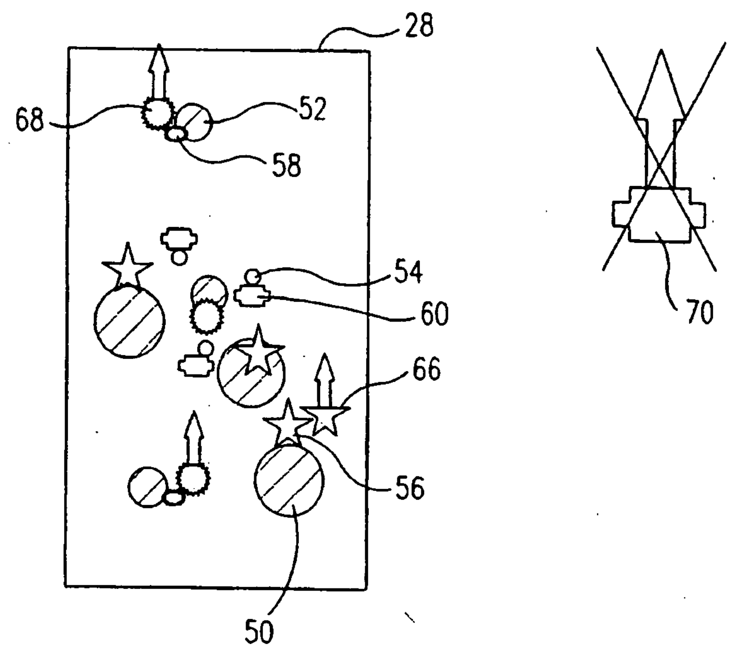

FIG. 3D

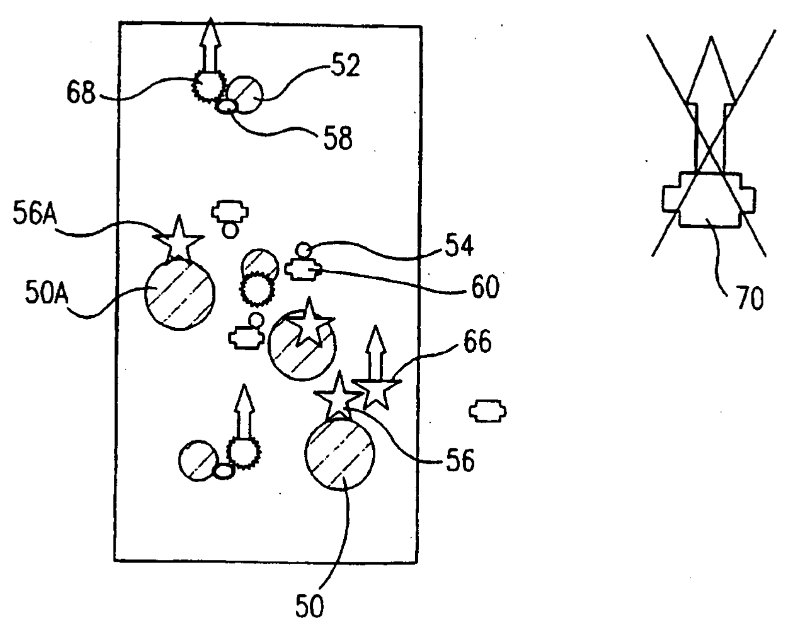

FIG. 4

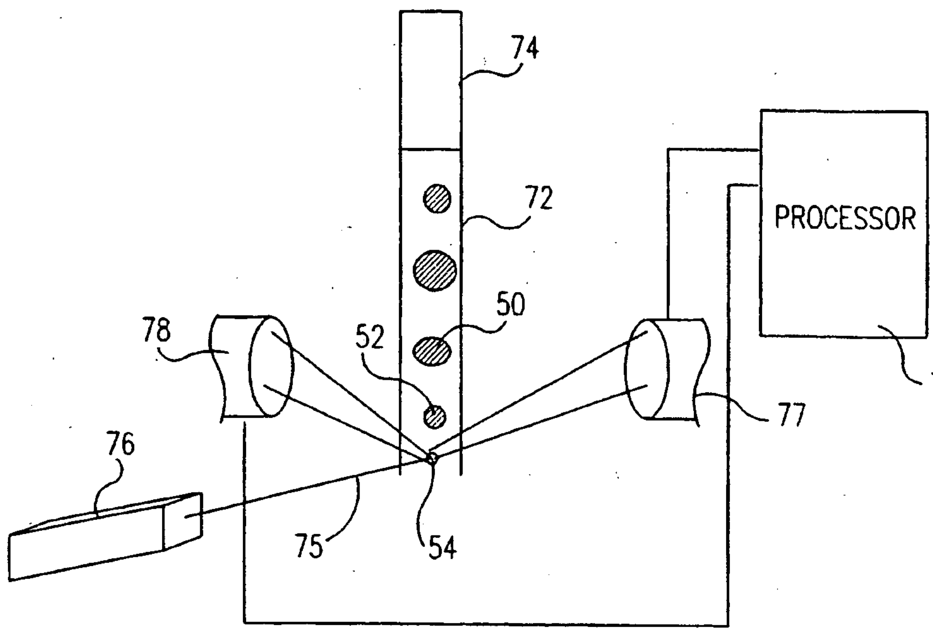

FIG. 5

WITH COLOR (+)	1	2	0
NO COLOR (−)	2	1	3
	SIZE 1	SIZE 2	SIZE 3

80

FIG. 6

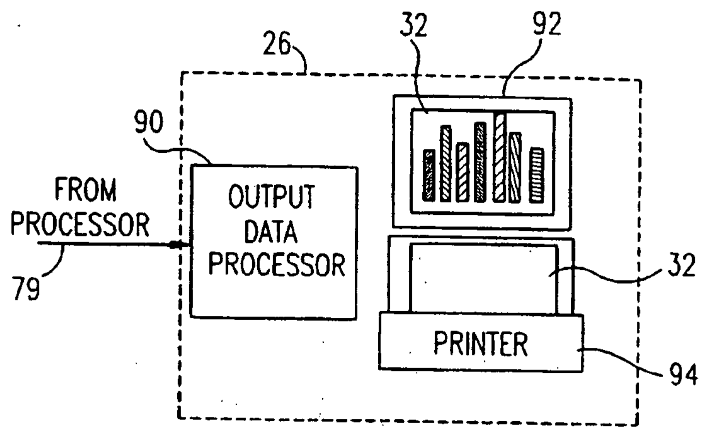

FIG. 7

PATIENT	HIV-1	HIV-2	HEPATITIS-B	HEPATITIS-C	HERPES SIMPLEX
#1	ZERO	ZERO	HIGH	ZERO	ZERO
#2	ZERO	HIGH	ZERO	ZERO	HIGH
#3	LOW	ZERO	ZERO	ZERO	ZERO
#4	ZERO	ZERO	ZERO	HIGH	LOW
#5	HIGH	ZERO	ZERO	ZERO	HIGH

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 97/00105

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/543 G01N33/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 17674 A (SIVENT A/S) 29 June 1995 see page 7, line 4 - line 8; claims 6,13 see page 15, line 20 - line 27 ---	1-7, 18-33
X	WO 94 28119 A (SMITHKLINE BEECHAM PLC) 8 December 1994 see claims 6-21 ---	8-17
A	EP 0 577 092 A (BECTON DICKENSON & COMPANY) 5 January 1994 cited in the application see the whole document -----	1-33

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
29 July 1997	04.08.1997

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IL 97/00105

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9517674 A	29-06-95	AU 1322095 A CA 2179823 A EP 0736177 A	10-07-95 29-06-95 09-10-96
WO 9428119 A	08-12-94	AU 6844694 A EP 0700434 A JP 8510380 T	20-12-94 13-03-96 05-11-96
EP 577092 A	05-01-94	US 5369036 A AU 4144093 A CA 2099433 A JP 7287016 A	29-11-94 06-01-94 03-01-94 31-10-95